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Energetic efficiency and temperature sensitivity of soil heterotrophic respiration vary with decadal-scale fire history in a wet sclerophyll forest

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ABSTRACT

Changes in fire regime and soil temperatures will be simultaneous symptoms of climate change in many regions around the world, yet very few studies have investigated how these factors will interact to affect soil carbon (C) cycling. Interacting effects of fire regime and temperature on soil C cycling processes might constitute an important but poorly-understood feedback to the global climate system. Using soils from one of the world's longest running prescribed fire trials in eastern Australia, we investigated the effect of fire regime on the rate, energetic efficiency, and temperature sensitivity of soil heterotrophic respiration and associated properties across a range of incubation temperatures (15 °C, 25 °C, and 35 °C). Levels of total, labile, soluble, and microbial biomass C were 32%, 59%, 64%, and 38% lower, respectively, in biennially-burned (2yB) soils than in soils that had not been exposed to fire since 1969 (NB soils). Moreover, while rates of heterotrophic respiration did not vary among NB, 2yB or quadrennially-burned (4yB) soils during the 55-day incubation period, values of qCO_2 (which are inversely related to microbial energetic efficiency) were 59.8% higher in 2yB soils than in NB soils. This suggests that biennial-burning is associated with soil conditions that promote energetic inefficiency in the microbial community and highlights the role of environmental stress as a determinant of respiratory responses to fire regime. Respiration temperature sensitivity (i.e. Q_{10} values) of 2yB soils was 86% greater than that of 4yB soils at the temperature range of 15–25 °C. This effect was absent at the temperature range of 25–35 °C and in soils to which labile C levels had been boosted through glucose addition. This pattern in Q_{10} values might be attributed to low quality soil organic matter in 2yB soils in combination with mechanisms associated with microbial community structure. Together these results enhance our understanding of C cycling in fire-affected soils and suggest a potentially important positive feedback between fire, climate change, and the terrestrial C cycle that warrants further investigation.

1. Introduction

Vegetation fires are affecting an increasingly large proportion of the Earth's surface due to the warmer air temperatures, altered seasonality and prolonged periods of drought associated with climate change (Alonso-Canas and Chuvieco, 2015; Westerling et al., 2011). Whether wild or prescribed, fires have strong potential to modify the stocks and cycling processes of soil carbon (C) (Muqaddas et al., 2015; Nave et al., 2011; Tilman et al., 2000). Given that soils contain around 1500 gigatonnes of C globally (Le Quéré et al., 2016), fire-induced changes to the nature and dynamics of soil C are likely to have profound implications for the global climate system. The respiration of CO_2 by soil micro-organisms (heterotrophic respiration; hereafter simply 'respiration') is particularly important in this context because it constitutes around 75% of total soil respiration and thus represents one of the largest global fluxes of C (Chapin et al., 2002; Raich and Potter, 1995).

Soil respiration responds to fire or heat in complex ways that vary based on fire regime and site characteristics. In recently or frequently burned areas, soil respiration rates tend to be depressed due to partial or complete sterilisation of topsoil (Hernandez et al., 1997; Holden and Treseder, 2013), or otherwise altered by changes in substrate quality and quantity (De Marco et al., 2005; O'Neill et al., 2006). On the other hand, long-term fire exclusion can constrain respiration by inducing microbial phosphorus (P)-limitation (e.g. Lagerström et al., 2009). Soil respiration rates are also governed by the 'efficiency' with which micro-organisms use soil organic C (Anderson and Domsch, 1985; i.e. 'energetic efficiency' or 'C use efficiency'). Microbial energetic efficiency is often approximated by the ratio of respired CO_2–C to microbial

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biomass C (referred to as 'the metabolic quotient' or 'qCO_2'; Anderson and Domsch, 1985). The metabolic quotient tends to be high when environmental conditions are unfavourable for, or imposing 'stress' upon, the microbial community, and can also be influenced by SOM quality and quantity, microbial community structure, and microbial dormancy (Anderson and Domsch, 1993; Wardle and Ghani, 1995). Given that differences in fire regime are often associated with distinct differences in any or all of these factors (e.g. Hernandez et al., 1997; González-Pérez et al., 2004; Shen et al., 2016), microbial energetic efficiency likely plays an important role in determining respiratory responses to fire regime. In particular, certain fire regimes can generate strong constraints on moisture, nutrients, or C (e.g. Lagerström et al., 2009; Muqaddas et al., 2015). Under such conditions micro-organisms might dedicate a greater proportion of energy to maintenance at the expense of growth (Odum, 1985), leading to higher rates of respiration per unit of microbial biomass (and thus higher qCO_2).

Furthermore, while many of the biological processes that drive C cycling are highly sensitive to temperature, it is unclear how the increases in ambient air and soil temperatures that define global warming will interact with changing fire regimes to modify soil C cycling processes. Such interactions could represent critical feedbacks to climate change (Davidson and Janssens, 2006), and understanding their nature will be essential for predicting the future trajectories of fire-climate-C relations and dynamics. To our knowledge, very few studies have investigated the effects of fire regime on the temperature sensitivity of soil respiration and associated properties and processes. Such effects seem probable because many of the determinants of respiration and respiration temperature sensitivity are influenced by fire regime. Vegetation and microbial community characteristics, soil moisture, soil elemental stoichiometry, and the concentrations, forms, and quality of soil organic matter (SOM) all influence respiration-temperature relationships (Cable et al., 2012; Fissore et al., 2013; Howard and Howard, 1993; Zechmeister-Boltenstern et al., 2015), and are all modified by changes in fire regime (Butler et al., 2018, 2017; Knicker et al., 2006; Noble and Slatyer, 1981; Shen et al., 2016).

While these factors are likely to interact and operate simultaneously, there is a particularly strong theoretical basis to expect that fire-induced changes in the nature of microbial substrate (i.e. soil C and SOM) will regulate the temperature sensitivity of respiration in fire-affected soils. Specifically, the 'enzyme-kinetic' hypothesis asserts that the decomposition of complex, recalcitrant organic compounds is inherently more temperature sensitive than that of simple, labile (i.e. those forms of SOM that are readily available to micro-organisms) organic compounds due to the larger number of enzymatic steps necessary to release a CO_2 molecule from the former compared to the latter (Ågren and Bosatta, 1996; Bosatta and Ågren, 1999). Under this framework, the number of enzymatic steps is inversely related to SOM 'quality', such that simple, labile SOM is considered to be of higher quality than complex, recalcitrant SOM. This theory is supported by empirical evidence (Fierer et al., 2006; O'Connell, 1990; Wagai et al., 2013), although some uncertainties remain regarding the timescales over which the quality of SOM is influential and the role of the soil mineral matrix in negating the importance of SOM quality (e.g. Wagai et al., 2013). At the same time, soils exposed to fire or high temperatures (hereafter 'fire-affected soils') tend to contain higher levels of recalcitrant, low quality organic matter than unheated soils (González-Pérez et al., 2004; Knicker, 2007; Rovira et al., 2012), and lower amounts of labile C (Butler et al., 2017; Muqaddas et al., 2015). Heated soils typically have high levels of complex 'pyrogenic' C forms (e.g. furans, phenols, carbonyls, and, in particular, aromatic polymers; Knicker, 2007). Depending on maximum temperature, heating can also stimulate dehydration, dehydrogenation, decarboxylation, and demethylation reactions in SOM, as well as the degradation of O-alkyl-C (Almendros et al., 1992; Baldock and Smernik, 2002; Knicker, 2007).

Given these observations, it seems reasonable that the temperature sensitivity of microbial respiration in fire-affected soils would tend to be greater than that of fire-protected soils due to higher levels of low quality, pyrogenic SOM in the former. This is further supported by the finding of Wagai et al. (2013) that the proportion of aromatic- and alkyl-C relative to O-alkyl-C in low density SOM, which should be high in fire-affected soils compared to unburned soils, is positively related to the short-term temperature sensitivity of CO_2 evolution. Indeed, in the enzyme-kinetic framework, aromatic- and alkyl-C can be regarded as of lower quality than O-alkyl-C (Wagai et al., 2013). Enhanced temperature sensitivity of respiration in fire-affected soils would constitute a positive feedback with implications for atmospheric CO_2 levels and thus climate. Nevertheless, very few studies have investigated this hypothetical effect and we are aware of none that have done so from the mechanistic perspective of enzyme-kinetic theory. Uribe et al. (2013) reported lower soil respiration temperature sensitivities after a wildfire; however, decadal-scale manipulations of fire regime will be necessary to adequately reflect the long-term consequences of climate change.

Thus, we carried out an incubation experiment using soils from a forty-five year-old prescribed forest fire trial in Peachester, southeastern Australia to investigate how fire regime affects soil respiration and the response of respiration and associated properties (labile and microbial biomass C, N and P, and the potential activities of C−, N− and P-acquiring enzymes) to changes in air temperature. We hypothesised that that different fire regimes produce different soil properties and, in particular, that soils exposed to recent, frequent fire have lower quality SOM (as indicated by lower labile C concentrations and labile: total C ratios) than soils that have not been exposed to recent, frequent fire. Thus, we also expected that heterotrophic respiration and microbial energetic efficiency would vary based on fire regime. Moreover, we expected that heterotrophic respiration of soils in recently, frequently burned areas would be more sensitive to temperature than that of soils with no recent history of fire exposure, due to the low-quality organic matter associated with the former. Finally, we predicted that, if fire regime regulates respiration temperature sensitivity by altering SOM characteristics, the addition of excess high-quality C in the form of glucose would negate the effects of fire regime on respiration temperature sensitivity.

2. Materials and methods

2.1. Study site and soil sampling

Surface soil samples (0–10 cm of mineral soil) were collected from the Peachester State Forest long-term prescribed fire experimental site (hereafter 'Peachester'; 26°52′S, 152°51′E) on the 25th of August 2017. Peachester is a wet eucalypt forest dominated by *Eucalyptus pilularis*, with Alfisol soils (Soil Survey Staff, 1999; red to yellow Kandosols in the Australian soil classification) that have a uniformly sandy texture until around 60 cm depth (Guinto et al., 2001). Precipitation at Peachester averages 1419 mm annually. Average daily temperatures range from a maximum of 30.3 °C in January to a minimum of 9.3 °C in June, with an overall daily average temperature of 20.7 °C (Bureau of Meteorology, 2018).

The prescribed burning experiment at Peachester has been running since 1969 and consists of three randomised, replicated fire regime treatments: burned every four years on average since 1972 (4yB), burned every two years on average since 1972 (2yB) and no burning since 1969 (NB). Burns at Peachester have been conducted in spring or winter and were generally patchy and of low intensity (i.e. < 2500 kW m^{-1}; Lewis et al., 2012). There were four 0.8 ha replicate plots for each fire frequency treatment at Peachester (twelve plots in total). In each of these plots we collected a single soil sample by hand using a small spade, with each sample consisting of five subsamples collected from a 10 × 10 m sampling area. Samples were collected from locations with similar microsite conditions. At the time of sampling, the most recent burns in both the 2yB and 4yB treatment plots had been in August 2013. The main limitation of this design is that

fire frequency is unavoidably confounded with time since fire for comparisons of 2yB and 4yB against NB, given that NB has remained unburned for around forty-eight years. Thus, while differences between 2yB and 4yB can be attributed to fire frequency, effects of quadrennial and biennial burning relative to fire exclusion should be attributed to fire 'regime' rather than fire frequency.

2.2. Analysis of initial soil properties

Soils were immediately sieved at 2 mm and then stored at 4 °C prior to the incubation experiment and analyses of initial soil properties. Soil water holding capacity was measured using the method described in Cassel and Nielsen (1986). Soil pH and EC were measured in a 1:5 soil: water solution. Soil total C and N were quantified using dry combustion with a Leco TCN Determinator (TruMac No. 830-300-400) after fine grinding (to < 15 μm) of the soil samples. Concentrations of labile C and N in soil were estimated using a hot water extraction (incubation in water at 70 °C for 16 h; Sparling et al., 1998). Total organic C and total N in the hot water extracts were measured via high-temperature catalytic oxidation using a Shimadzu TOCN analyser (Chen et al., 2005).

Microbial biomass C, N, and P (MBC, MBN, and MBP respectively) were measured using the chloroform fumigation method (Brookes et al., 1985, 1982; Vance et al., 1987). We used 0.5M K_2SO_4 to extract C and N and 0.5M $NaHCO_3$ to extract P in the fumigated and corresponding non-fumigated samples. Concentrations of organic C and total N in K_2SO_4 extracts was measured with high-temperature catalytic oxidation, while the concentration of PO_4^{3-} in $NaHCO_3$ extracts was measured using molybdenum-blue spectrophotometry (Murphy and Riley, 1962). Concentrations of soil MBC, MBN, and MBP were then calculated using the conversion factors of 2.64, 2.22, and 0.4 respectively (Brookes et al., 1985, 1982; Vance et al., 1987). Concentrations of organic C, total N, and PO_4^{3-} in the extracts from the non-fumigated samples were used as a measure of soil soluble organic C (SOC), soluble total N (STN), and soluble PO_4^{3-} (SP) contents (Toberman et al., 2014). The potential activities of three extra-cellular enzymes that hydrolyse C (β-glucosidase or 'BG'), N (chitinase or 'CHN'), and P (acid phosphatase or 'AP') were assayed using p-nitrophenol spectrophotometric methods (Eivazi and Tabatabai, 1988, 1977; Tabatabai and Bremner, 1972). The Sigma codes for the substrates used were N7006 for BG, N9376 for CHN, and P4744 for AP.

2.3. Soil incubation experiment and subsequent chemical analyses

The incubation experiment was established on the 1st of September 2017. This experiment consisted of the following factors: fire regime (levels of NB, 4yB, and 2yB), incubation temperature (with levels of 15 °C, 25 °C, and 35 °C), and glucose amendment (levels of no amendment [hereafter 'non-amended' samples] and 40 mg glucose g^{-1} oven-dry soil [hereafter 'glucose-amended' samples). Glucose treatments were used to indirectly assess the role of fire regime-altered SOM quality on respiration temperature sensitivity. Specifically, we predicted that, if recent, frequent fire reduces SOM quality and consequently increases temperature sensitivity, then the addition of glucose (a highly-labile form of C) should eliminate any such effects of fire regime. Thus, in the context of our study, glucose amendments can be thought of as controls for the effect of C lability, as modified by fire regime, on soil respiration. Each treatment combination consisted of four replicates, corresponding directly to the four fire regime replicate plots at the Peachester field trial. Thus, there were 3 × 3 × 2 × 4 = 72 incubation samples in total. We retained the Peachester field replicates in order to maintain the spatial variability that exists among the replicate plots.

Fifteen grams of fresh, field-moist soil were added to 70 ml polypropylene jars (Sarstedt, Germany). These jars were then placed into larger glass mason jars and pre-incubated at 15 °C for 14 days under aerobic conditions. After pre-incubation, glucose was added using a concentrated glucose solution. Deionised water was then added to all soil samples to bring the moisture levels to 50% of water holding capacity, and the samples were gently mixed with a stainless-steel spatula to ensure even moisture distribution. Sodium hydroxide (NaOH) traps, which consisted of 70 ml polypropylene jars that contained 10 ml of 0.1M NaOH, were added to each mason jar. Mason jars were then sealed and placed into one of three incubators which were set at 15 °C, 25 °C, and 35 °C, respectively.

The soils were incubated for 55 days, with measurements of cumulative CO_2—C evolution made on days 1, 2, 3, 4, 5, 6, 7, 8, 16, 23, 30, 31, 37, 44, 45, 51, and 55 for the non-amended samples, and on days 1, 2, 3, 4, 5, 6, 7, 8, 31, 45, and 55 for the glucose-amended samples. For non-amended samples the CO_2 trapping period was continuous from day 0–55, but for glucose-amended samples the CO_2 trapping period was only for the 24 h preceding each CO_2 measurement time. The respired CO_2 in each NaOH trap was precipitated as $SrCO_3$ by addition of a saturating quantity of $SrCl_2$ (Biasi et al., 2005). The remaining NaOH was then titrated back with 0.1M HCl, enabling calculation of the amount of CO_2 respired. At the end of the incubation experiment, MBC, MBN, MBP, soluble C, N and P, and the potential activities of BG, CHN and AP were measured again in all soil samples using the methods described above.

2.4. Data preparation and statistical analyses

The metabolic quotient (qCO_2) of soil respiration was calculated as soil respiration rate per unit of MBC (reported as μg CO_2—C mg MBC^{-1} $hour^{-1}$). Metabolic quotient was only calculated for non-amended samples. We used MBC of initial (pre-incubation) soil samples to calculate qCO_2. Day 2 data were used for calculation of qCO_2 because twenty-two out of thirty-six day 1 respiration measurements of non-amended samples were below detection limits and thus designated a value of 0 μg CO_2—C g $soil^{-1}$ $hour^{-1}$. The temperature sensitivity of soil respiration was quantified using the Q_{10} temperature coefficient, which expresses the rate of change of respiration in response to a 10 °C increase in temperature:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10^\circ}{(T_2 - T_1)}} \quad (1)$$

where R_1 and R_2 are the rates of respiration for the lower and higher temperature, respectively, and T_1 and T_2 are the lower and higher temperatures, respectively. Thus, we had three sets of Q_{10} values: a low temperature set (for the 15–25 °C temperature range), a high temperature set (for the 25–35 °C temperature range), and an overall set (for the 15–35 °C temperature range). Day 1 measurements were excluded from calculation and subsequent analysis of Q_{10} because forty-one out of the total seventy-two day 1 respiration measurements were below detection limits.

According to a global meta-analysis conducted by Hamdi et al. (2013), soil respiration Q_{10} values have ranged between 0.5 and 6.5 (mean = 2.21; n = 552) in studies where the average of the upper and lower experimental temperatures was between 15 °C and 35 °C. Across our entire Q_{10} data set (excluding day 1, when respiration was below detection levels for 56.9% of the samples), the mean Q_{10} = 3.62 for non-amended samples (n = 576) and 3.28 for glucose-amended samples (n = 360). In our dataset, 84.5% of non-amended sample Q_{10} values and 80.6% of glucose-amended sample Q_{10} values fell within the range reported by Hamdi et al. (2013). However, Q_{10} values ranged from 0.02 to 169.4 for non-amended samples and from 0.02 to 34.6 for glucose-amended samples. We considered several of the higher Q_{10} values to be unreasonably high, due to experimentalist error, and thus employed the following method to detect and remove extreme outliers. First, we pooled our data with that of Hamdi et al. (2013). This was done separately for non-amended and glucose-amended samples, resulting in two datasets that contained 1128 and 912 observations,

respectively. Outlier cut-off values were then calculated for each dataset as the overall mean + three standard deviations (Encyclopedia of Mathematics, 2011). Observations that exceeded this cut-off were excluded from subsequent statistical analyses. Nine out of 576 non-amended sample observations (= 1.56%) and 20 out of 360 glucose-amended sample observations (= 5.56%) were excluded based on this approach. We then imputed the missing values using means of the remaining replicates or observations for a given measurement time. We recognise that mean substitution is a less-than-ideal approach to imputation, and its potential to induce bias should be considered when interpreting our Q_{10} data (Donders et al., 2006). However, one advantage of this method is that it does not change the mean of the particular treatment combination. This is important in our study because we were focused primarily on means rather than relationships among variables. Moreover, when only a small number of values are missing, the use of single imputation methods (e.g. mean imputation), as opposed to more sophisticated multiple imputation methods, is unlikely to have strong effects on estimates of variance and can, therefore, be acceptable in some circumstances (Sainani, 2015).

One-way ANOVAs were used to test for differences in the properties of initial soil samples among the Peachester fire regime treatments. Two-way factorial ANOVA was used to assess the effects of fire regime and incubation temperature on qCO_2 values. Repeated measures ANOVAs were used to evaluate the effect of fire regime on the rate and temperature sensitivity (Q_{10}) of soil respiration throughout the incubation period. Tukey's Honestly Significant Difference (HSD) was used to compare treatment means where ANOVA P-values were < 0.05. The qCO_2 data were log-transformed prior to analyses because Tukey's HSD test is sensitive to large differences in population variances and there were large differences in qCO_2 variance among incubation temperatures (variance before log transformation: 15 °C = 0.23, 25 °C = 2.20, 35 °C = 97.3; variance after log transformation: 15 °C = 0.27, 25 °C = 0.15, 35 °C = 0.08).

Non-amended and glucose-amended samples were analysed separately. However, we also performed the analyses with non-amended and glucose-amended samples pooled together (excluding collection days that only occurred in one set) to evaluate the effect of glucose amendment and its interaction with fire regime. We used factorial ANOVAs to analyse the effects of fire regime and incubation temperature on cumulative respiration (of non-amended samples only), soluble and microbial biomass C, N and P concentrations, and the potential activities of BG, CHN and AP.

3. Results

3.1. Initial soil properties

Fire regime had numerous significant effects on the properties of our initial soil samples (Table 1). Soils from the 4yB treatment had significantly higher water holding capacity than soils from the NB and 2yB treatments, while 2yB soils had significantly lower moisture content than NB and 4yB soils at the time of sampling. Soils from the NB treatment had significantly higher levels of labile (i.e. hot water-extractable) organic C and microbial biomass C, as well as higher SOC to total C ratios (SOC:TC), than soils from the 2yB treatment. Soils from the NB treatment had higher levels of SOC than 4yB soils, which in turn had higher SOC levels than 2yB soils. Concentrations of total, labile, and soluble total N were higher in NB than 2yB soils. The potential activities of AP were significantly lower in soils from the 2yB treatment than those from the NB treatment. Fire treatment had no significant effects on the pH, EC, total C, SP, MBN, MBP, BG activity or CHN activity of initial soil samples (Table 1). The effects of fire regime on the levels of various pools of C, N, and P in soil translated to numerous significant effects on soil C:N:P stoichiometric ratios (Table 1). Total C:N ratios were lower in NB soils than in 2yB soils. On the other hand, SOC:STN, SOC:SP, and STN:SP ratios were all significantly higher in NB soils than 2yB soils, as were microbial biomass C:P and N:P ratios. There were no significant differences in labile OC:N ratios, microbial biomass C:N ratios, or eco-enzymatic ratios among fire regime treatments.

3.2. Dynamics, efficiency, and temperature sensitivity of respiration in fire-affected soils

The effects of measurement time were significant in many of the repeated measures ANOVAs (see Supplementary Tables); however, here we focus on the effects of fire regime and fire regime × temperature interactions, as these factors were our primary interest. Fire regime treatment had no significant effect on respiration rates of non-amended (Fig. 1a—c) or glucose-amended samples or on the cumulative respiration of the former (Tables S1—3). Incubation temperature had a significant and positive effect on soil respiration rate for both non-amended and glucose-amended samples (Tables S1 and S2). Predictably, respiration rates were greatest under incubation at 35 °C and lowest under incubation at 15 °C; however, there was no significant difference in cumulative respiration between the 35 °C and 25 °C incubation temperatures (15 °C mean = 690 μg CO_2—C g soil; 25 °C mean = 1834 μg CO_2—C g soil; 35 °C mean = 2048 μg CO_2—C g soil; Tukey's HSD critical value for comparison = 458; Table S3). Across all non-amended samples, respiration rates were highest on day two of the incubation and showed a second significant peak on or around day 23 (Fig. 1). This second peak in respiration was most pronounced for samples incubated at 15 °C and least pronounced for samples incubated at 35 °C. Indeed, for samples incubated at 15 °C, the day 23 peak was markedly higher than the day 2 peak.

Soil qCO_2 values on day 2 of the incubation were significantly affected by fire regime ($P = 0.019$) and incubation temperature ($P < 0.001$; Fig. 2; Table S4). Values of qCO_2 were 59.8% higher, on average, for 2yB soils than NB soils, and increased with temperature from 1.1 at 15 °C, 3.0 at 25 °C, 33.2 at 35 °C. The respiration rates of 2yB non-amended soils were significantly more sensitive to temperature than those of 4yB non-amended soils; however, this effect was only present for low temperature Q_{10} (Fig. 3a; Table S5—7). Average values of low temperature Q_{10} from days 2–55 were 5.60 for 2yB soils, 4.14 for NB soils, and 3.01 for 4yB soils. Values of high and overall Q_{10} were not significantly affected by the fire-exposure history of soil samples (Fig. 3b; Tables S6 and S7). For glucose-amended soils samples, fire regime had no effect on respiration Q_{10} (Table S8—10). Consistent with these findings, low temperature Q_{10} was significantly affected by the interaction between glucose amendment condition and fire regime when non-amended and glucose-amended samples were analysed together (Table S11).

3.3. Soil properties after incubation

Incubation temperature had marked effects on numerous chemical and stoichiometric properties of non-amended soils (Fig. 4 and S1). Microbial biomass C and C:P ratios were depressed in samples that were incubated at 35 °C relative to samples incubated at 15 °C (Fig. 4b,e). Samples incubated at 15 °C and 25 °C had significantly higher BG activities (Fig. 4c), MBN concentrations, BG activities, soluble C:N ratios, and BG:CHN ratios than samples incubated at 35 °C (Fig. 4c and S1b,g,j), but also had significantly lower soluble N concentrations and soluble N:P ratios (Figs. S1a and i).

In general, the effects of fire regime on non-amended soil properties at the end of the incubation period were similar to those observed in pre-incubation samples (Fig. 4 and S1). However, after 55 days of incubation, 4yB soils also had higher levels of MBN than 2yB soils and higher levels of MBP than NB soils (Figs. S1b and e). Microbial biomass C:N ratios were also significantly higher in NB soil than 2yB soils after incubation (Fig. 4d). Soils from the 2yB treatment had lower BG, CHN, and AP activities than NB and 4yB soils (Fig. 4c and S1c,f), as well as higher BG:AP and CHN:AP ratios (Fig. S1k,l), at the end of the

Table 1
Initial properties of surface soil samples (0–10 cm) collected from the unburned (NB), quadrennially-burned (4yB), and biennially-burned (2yB) treatments at the Peachester State Forest prescribed burning experiment (Queensland, Australia) in August 2017.

Soil property[a]	NB	4yB	2yB	F-statistic$_{2,9}$ (P-value)
Field moisture (%)	14.7 (± 1.3)^{A}	15.2 (± 0.9)^{A}	9.2 (± 1.1)^{B}	8.8 (0.008)
WHC (%)	47.7 (± 4.0)^{A}	60.9 (± 1.8)^{B}	38.6 (± 3.6)^{A}	11.6 (0.003)
pH	4.16 (± 0.20)	4.36 (± 0.12)	4.86 (± 0.22)	3.8 (0.063)
EC (μS cm^{-1})	35.7 (± 2.6)	47.2 (± 13.9)	28.1 (± 2.2)	1.7 (0.308)
Total C (%)	6.20 (± 0.71)	6.27 (± 0.47)	4.22 (± 0.62)	3.7 (0.068)
Total N (%)	0.26 (± 0.04)^{A}	0.27 (± 0.02)^{A}	0.13 (± 0.02)^{B}	7.8 (0.011)
Total C:N	24.2 (± 1.1)^{A}	23.4 (± 0.5)^{A}	31.8 (± 1.4)^{B}	19.5 (< 0.001)
Labile C (mg kg^{-1})	656 (± 63.7)^{A}	488 (± 124)^{AB}	271 (± 47)^{B}	5.2 (0.032)
Labile N (mg kg^{-1})	45.7 (± 3.6)^{A}	36.1 (± 8.8)^{AB}	21.3 (± 2.3)^{B}	4.7 (0.039)
Labile C:N	14.3 (± 0.4)	13.4 (± 0.4)	12.5 (± 1.3)	1.3 (0.317)
Labile C: Total C	107 (± 8.7)	79 (± 20.6)	69 (± 15.8)	1.6 (0.255)
Soluble C (mg kg^{-1})	153 (± 13.6)^{A}	116 (± 6.8)^{B}	55 (± 5.1)^{C}	28.5 (< 0.001)
Soluble N (mg kg^{-1})	14.8 (± 1.3)^{A}	11.7 (± 0.8)^{A}	6.6 (± 0.5)^{B}	19.2 (< 0.001)
Soluble PO_4^{3-} (mg kg^{-1})	3.98 (± 0.36)	4.48 (± 0.38)	4.48 (± 0.58)	0.4 (0.684)
Soluble C:N	10.4 (± 0.4)^{A}	9.9 (± 0.2)^{A}	8.2 (± 0.2)^{B}	17.6 (< 0.001)
Soluble C:P	39.3 (± 4.8)^{A}	26.6 (± 3.1)^{AB}	13.3 (± 2.8)^{B}	12.4 (0.003)
Soluble N:P	3.80 (± 0.5)^{A}	2.70 (± 0.4)^{AB}	1.60 (± 0.3)^{B}	8.3 (0.009)
Soluble C: Total C	24.9 (± 0.9)^{A}	18.7 (± 1.3)^{B}	13.6 (± 1.7)^{B}	17.9 (< 0.001)
MB C (mg kg^{-1})	681 (± 37.9)^{A}	602 (± 95.2)^{AB}	419 (± 43.6)^{B}	4.4 (0.048)
MB N (mg kg^{-1})	77.2 (± 6.2)	72.6 (± 9.6)	53.3 (± 4.9)	3.1 (0.095)
MB P (mg kg^{-1})	38.9 (± 4.2)	41.5 (± 2.6)	37.8 (± 5.7)	0.2 (0.472)
MBC:MBN	8.87 (± 0.25)	8.21 (± 0.3)	7.84 (± 0.2)	3.5 (0.075)
MBC:MBP	17.9 (± 1.3)^{A}	14.3 (± 1.4)^{AB}	11.5 (± 0.9)^{B}	6.9 (0.015)
MBN:MBP	2.01 (± 0.09)^{A}	1.73 (± 0.13)^{AB}	1.47 (± 0.15)^{B}	4.7 (0.041)
BG (μg PNP g^{-1} hr^{-1})	40.8 (± 10.0)	47.8 (± 10.0)	25.8 (± 8.0)	1.4 (0.289)
CHN (μg PNP g^{-1} hr^{-1})	18.8 (± 1.4)	18.5 (± 4.8)	9.6 (± 1.3)	3.2 (0.092)
AP (μg PNP g^{-1} hr^{-1})	691 (± 122)^{A}	559 (± 123)^{AB}	255 (± 59)^{B}	4.5 (0.045)
BG:CHN	2.12 (± 0.48)	2.73 (± 0.27)	2.84 (± 0.93)	0.4 (0.688)
BG:AP	0.056 (± 0.006)	0.097 (± 0.026)	0.099 (± 0.029)	1.1 (0.365)
CHN:AP	0.030 (± 0.005)	0.037 (± 0.01)	0.042 (± 0.009)	0.6 (0.588)

[a] WHC = water holding capacity; EC = electrical conductivity; C = carbon; N = nitrogen; P = phosphorus; soluble = hot-water-extractable forms; OC = organic carbon; MB = microbial biomass; BG = potential β-glucosidase activity; CHN = potential chitinase activity; AP = potential acid phosphatase activity; PNP = *p*-nitro-phenol.

incubation period. Of all soil properties measured, only microbial biomass N:P ratio was significantly affected by the interaction between fire regime and incubation temperature (Fig. 4f). Specifically, microbial biomass N:P was significantly lower in samples that had been incubated at 35 °C, relative to samples incubated at 25 °C and 15 °C, but only in NB soils. Further, microbial biomass N:P was higher in NB soils than in 2yB and 4yB soils, but only for samples incubated at 25 °C.

4. Discussion

Numerous studies from a variety of ecosystems reveal that fire has complex and varied effects on soil respiration (e.g. Czimczik et al., 2006; Michelsen et al., 2004; Richards et al., 2012; Wütrich et al., 2002). Previously reported effects include both increases and decreases in rates of CO_2 evolution at a range of timescales in the post-fire environment from hours to decades (e.g. Lagerström et al., 2009; Michelsen et al., 2004; Wütrich et al., 2002). Meta-analyses suggest that these effects are largely a direct result of soil sterilisation during burns and thus coupled to the subsequent rate of microbial community recovery (Dooley and Treseder, 2011). We found that initial concentrations of MBC were on average 38% higher in NB soils than in 2yB soils (Table 1); however, there were no significant differences among fire regime treatments in the overall rates of soil heterotrophic respiration or in the cumulative amount of CO_2 respired.

Thus, these findings enhance our understanding of C cycling under contrasting fire regimes by confirming the role of microbial energetic efficiency, or inefficiency, as a driver of respiratory responses to fire regime. This is demonstrated by qCO_2 values on day 2 of the incubation, which were 59.8% higher in 2yB soils than in NB soils (Fig. 2). According to Wardle and Ghani (1995), qCO_2 can give insight into the suitability or adversity of environmental conditions for microorganisms. In this sense, variation in qCO_2 might reflect different levels of microbial energetic 'stress' associated with the different fire regimes at Peachester, with high qCO_2 indicating low energetic efficiency and, potentially, high microbial stress (Wardle and Ghani, 1995). Such stress might have arisen in the 2yB treatment for several reasons, including, but not limited to, the lower levels of moisture, C, and N, as well as the lower soluble C: total C ratios and higher total C:N ratios, associated with biennially-burned soils (Table 1). Thus, the responses of soil heterotrophic respiration to changes in fire regime are likely regulated not only by microbial sterilisation and changes in the degree of nutrient limitation (e.g. Lagerström et al., 2009), but also by changes in the overall suitability of environmental conditions for the microbial community.

However, variation in qCO_2 values could also reflect differences in microbial community composition. Fires tend to have more severe negative impacts on fungi than bacteria (Bååth et al., 1995; Pietikaeinen and Fritze, 1995; Shen et al., 2016), and fungal to bacterial biomass ratios have been shown to be negatively related to qCO_2 (Sakamoto and Oba, 1994). The low microbial biomass N:P ratios in 2yB soils (Table 1) could reflect higher bacterial biomass relative to fungal biomass (Cleveland and Liptzin, 2007; Reiners, 1986; Sterner and Elser, 2002; Zhang and Elser, 2017), although it is unclear whether microbial biomass N:P is a useful indicator of bacterial : fungal biomass ratios (Mouginot et al., 2014). Moreover, Wardle and Ghani (1995) noted that recalcitrant organic matter tends to be associated with high qCO_2 values. Thus, qCO_2 values could be higher in fire-affected soils, regardless of microbial stress or community composition. Whatever the cause, our results indicate that 2yB soils evolved more CO_2 for every unit of microbial biomass than NB soils, and that the effects of fire regime on qCO_2 can be detected up to four years after the most recent fire in the 2yB treatment (Fig. 2). A sustained inefficiency of microbial C use could

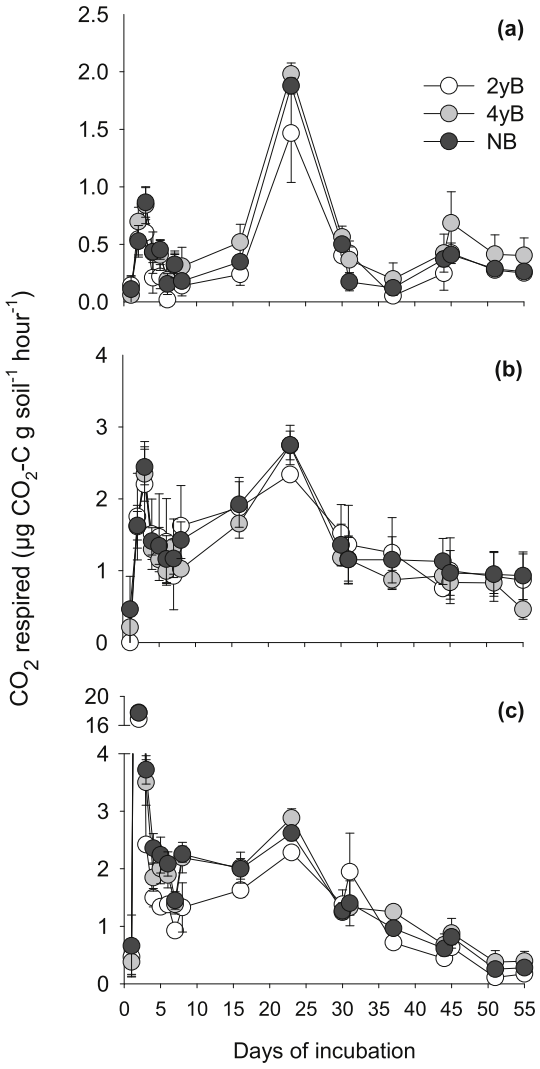

Fig. 1. Rates of soil CO_2 respiration of non-amended samples compared among fire regimes under incubation at (a) 15 °C, (b) 25 °C, and (c) 35 °C (n = 4 for each fire regime × temperature treatment combination).

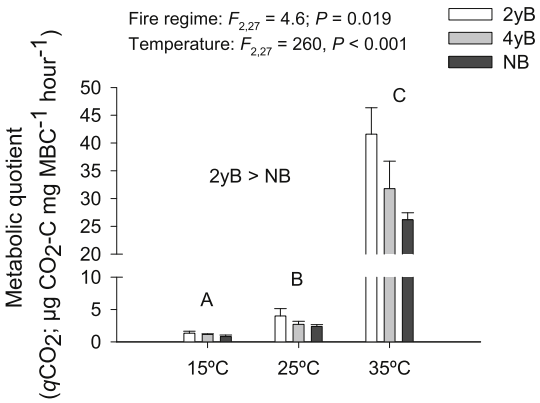

Fig. 2. Metabolic quotient of soil microbial biomass (qCO_2) in non-amended soil samples on day 2 of the incubation period; Tukey's HSD post-hoc comparison results shown for fire regime and temperature, with different upper case letters indicating significant differences between incubation temperature means at $P < 0.05$; 2yB = biennially-burned treatment, 4yB = quadrennially-burned treatment, and NB = unburned treatment.

have contributed to the low levels of labile, soluble, and total C in 2yB soils relative to NB soils (Table 1). However, fire-induced losses of soil C are often attributed primarily to direct volatilisation of soil C during combustion (González-Pérez et al., 2004; Muqaddas et al., 2015). Thus, ongoing study into effects of fire regime on microbial C use efficiency is warranted. The importance of such study is underscored by the consistency of fire regime's effect on qCO_2 across the range of temperatures in our study (Fig. 2), as this suggests that the effects are likely to endure under a warming climate.

Our Q_{10} data further support the view that ongoing and future changes in fire regimes might have consequences for soil respiratory responses to climate warming. We expected that the temperature sensitivity of CO_2 respiration would be greatest in the most severely fire-affected soils and lowest in long-unburned soils. Our results were partially consistent with this hypothesis, as low temperature Q_{10} values were 86% higher in non-amended soils from the 2yB treatment than non-amended soils from the 4yB treatment ($P = 0.037$; Fig. 3a). The magnitude of this response was largely due to measurements taken on days 6, 8, 16, and 37. However, Q_{10} did not differ significantly between 2yB and NB soils (Fig. 3a), and was not affected by fire regime at the 25–35 °C or 15–35 °C temperature ranges (Fig. 3b; Table S7). We based our prediction on the tendency for fires to generate pyrogenic SOM and to reduce the concentration of labile C forms, because enzyme-kinetic theory asserts that the decomposition of complex, recalcitrant (i.e. low quality) SOM has inherently greater temperature sensitivity than that of labile (i.e. high quality) forms (Bosatta and Ågren, 1999). The role of SOM quality was supported by our finding that the addition of glucose negated any effects of fire regime on respiration Q_{10} (Tables S5 and S11). However, our glucose-amendment test could not isolate the influence of SOM quality from that of overall soil C quantity. Moreover, while ratios of soluble C to total C were lower in 2yB soils than 4yB soils, they were also lower than those in NB soils (Table 1). Thus, while likely important, SOM quality cannot be the only property that regulates the effects of fire regime on soil heterotrophic respiration Q_{10} at Peachester.

Indeed, microbial community composition might also have contributed to the patterns of temperature sensitivity in our experiment. For instance, high microbial biomass N:P ratios in NB soils at 25 °C (Fig. 4f) might reflect high fungal : bacterial biomass ratios (Cleveland and Liptzin, 2007). Given that fungal activity tends to be more sensitive to temperature than that of bacteria (Alster et al., 2018), temperature-driven increases in the dominance of fungi over bacteria in NB soils might have increased NB low temperature Q_{10}. This could explain the similarity in low temperature Q_{10} values between NB and 2yB soils despite their significant differences in soil C quality and quantity (Table 1). It is unclear what facilitated the temperature-driven stoichiometric restructuring of microbial biomass in NB soils, but their high levels of C and N and high-quality organic matter might have had some influence (Table 1; Fig. 2).

Our findings related to respiration Q_{10} reveal a potentially important yet seemingly overlooked feedback to climate change, where changes in fire regime can alter or, in at least some cases, enhance the sensitivity of soil heterotrophic CO_2 respiration to rising air and soil temperatures. On the other hand, Uribe et al. (2013) reported that respiration Q_{10} values were lower in soils that had been exposed to wildfire than in soils from adjacent unburned areas. Interestingly, Uribe et al. (2013) suggested that this effect might have been caused by increased levels of recalcitrant SOM content in the wildfire-affected soils. Our findings, along with enzyme-kinetic theory, suggest that this is unlikely to be the case. Further study is warranted to reconcile our findings with those of Uribe et al. (2013), and the differences between our studies can provide a guide for future work. For instance, Uribe et al. (2013) studied the effects of 'one-off' wildfire events, while our study investigated the effects of decadal-scale prescribed burning. Differences in fire regime likely had a strong influence over our respective findings, given that wild and prescribed fire differentially affect soil C

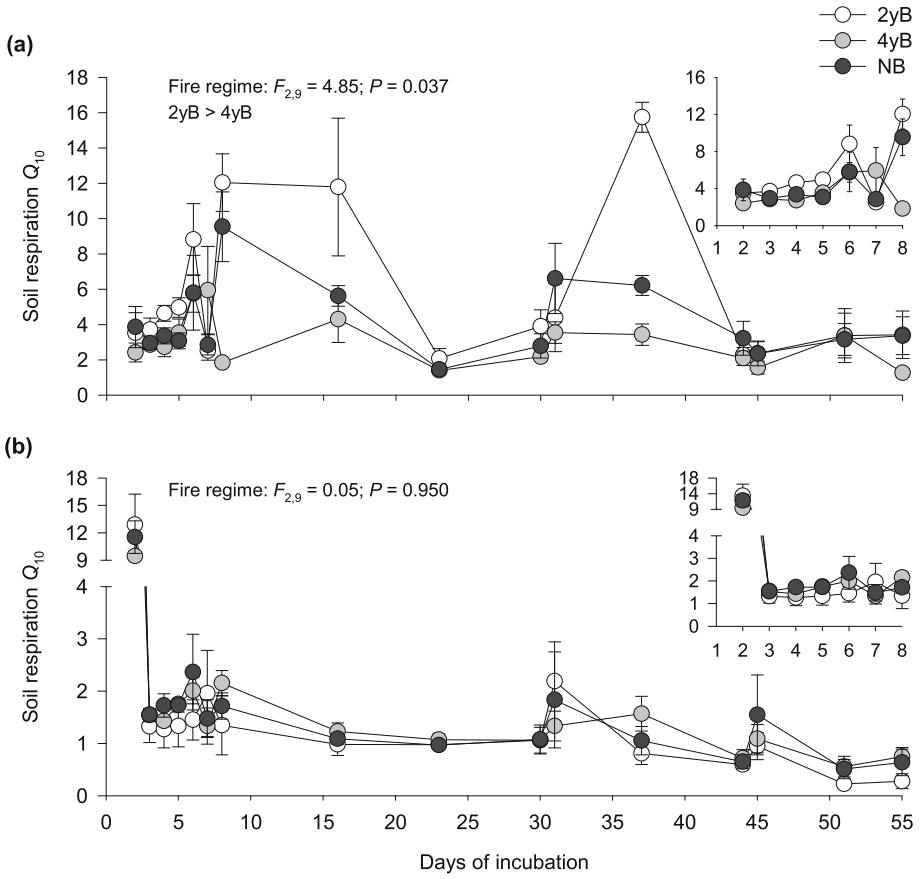

Fig. 3. Temperature sensitivity of soil respiration (Q_{10}) over 55 days compared among fire regime treatments for increases in temperature from (a) 15 °C–25 °C and (b) 25 °C–35 °C. Insets focus on days 2–8 of the incubation period. Results of repeated measures ANOVA provided for fire regime treatment effects.

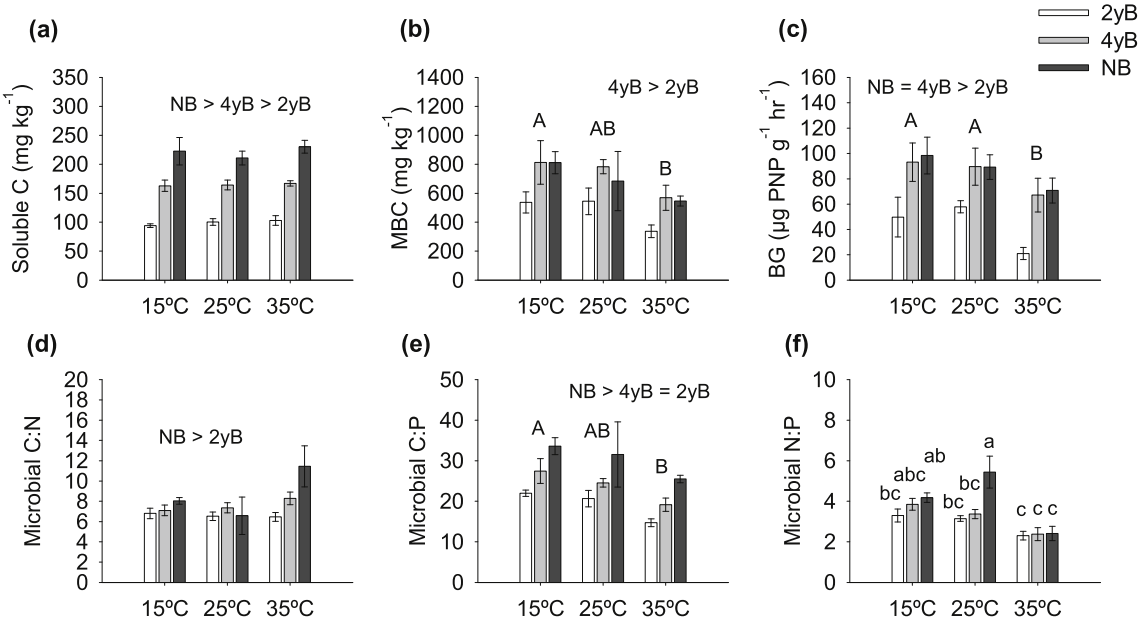

Fig. 4. Means (± standard errors) of soil (a) soluble carbon (C), (b) microbial biomass C, (c) potential β-glucosidase (BG) activity, (d) microbial biomass C: nitrogen (N) ratio, (e) microbial biomass C: phosphorus (P) ratio, and (f) microbial biomass N:P ratio compared among fire regime (biennially-burned [2yB], quadrennially-burned [4yB], and unburned [NB]) and temperature (15 °C, 25 °C, and 35 °C) treatment combinations after 55 days of incubation; results from Tukey's HSD post-hoc comparison of treatment means shown where factorial ANOVA P-values were < 0.05, with upper case letters used to compare incubation temperature means and lower case letters used to compare the fire regime × incubation temperature treatment combination means.

and nutrients (Butler et al., 2018; Nave et al., 2011). The wider range of temperatures attained during wildfires compared to prescribed burns likely increases the variety of forms and quality of pyrogenic SOM generated (Knicker, 2007), although it is not yet clear how this might affect Q_{10} values. Additionally, by using an incubation approach we captured the effects of fire regime on heterotrophic respiration only, while the field-based study of Uribe et al. (2013) focused on heterotrophic plus autotrophic respiration. It is unlikely that soil C quality and content would affect autotrophic respiration Q_{10} in the same manner as heterotrophic respiration Q_{10}. Finally, the differences in numerous environmental factors including vegetation, soil type, rainfall and daily temperature will almost certainly have contributed to the differences between our results and those of Uribe et al. (2013).

Thus, future research will need to encompass a variety of fire regimes across a broad range of sites if it is to disentangle the complex influences of fire history and environmental conditions on soil respiration. This is highlighted by our finding that fire regime only affected respiration Q_{10} at lower temperatures (i.e. 15–25 °C; Fig. 3; Tables S5—7). Given that temperate and boreal forest soils generally contain larger amounts of C than tropical or subtropical forest or grassland soils, are highly sensitive to warming (Goulden et al., 1998; Karhu et al., 2016), and are increasingly affected by fire (Johnstone et al., 2010), it is important that future studies include fire-affected sites at high latitudes.

These results are the first that show how the temperature sensitivity of soil heterotrophic respiration can vary based on a soil's decadal-scale history of fire exposure. In this regard, our study supports previous findings from Peachester that indicate that quadrennial prescribed burning is likely to be more sustainable than biennial prescribed burning (e.g. Muqaddas et al., 2015; Toberman et al., 2014). Moreover, it has been argued that the use of a single Q_{10} value in C models is unreasonable due to the potentially large variability in Q_{10} (e.g. Fierer et al., 2006). Our findings support and extend upon this by showing that heterogeneity of fire history within landscapes could contribute to spatial variation in Q_{10}, and that changes in fire regime over time have potential to modify Q_{10} at a given location. Unravelling the mechanisms behind these effects will be essential to predicting soil respiratory responses to the rising temperatures and changing patterns of fire behaviour worldwide.

5. Conclusions

This long-term prescribed fire study has clearly demonstrated that concentrations of labile, soluble, and microbial biomass C were lower in 2yB soils than in NB soils, as were soluble : total C ratios and total, labile, and soluble N concentrations. However, soil respiration rates did not differ among NB, 2yB or 4yB soils during a 55-day incubation trial. This corresponded with values of qCO_2 that were 59.8% higher in 2yB soils than in NB soils, which suggests that unfavourable environmental conditions in 2yB soils induced energetic inefficiency or 'stress' in the microbial community. Thus, changes in microbial C use efficiency likely influence respiratory responses to shifts in fire regime and temperature. The respiration rates of 2yB soils were significantly more temperature sensitive than those of 4yB soils, but not those of NB soils. This effect was only significant for the 15–25 °C temperature range and might, therefore, prove particularly important during cooler periods of the year. Moreover, glucose addition negated this effect, suggesting that soil C quality or quantity plays a role in regulating the effects of fire regime on Q_{10}. These results improve our understanding of C cycling in fire-affected soils and highlight the need for further investigations into the potential positive feedbacks between fire, climate change, and the terrestrial C cycle.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.03.022.

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